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Short communication

Polyclonals to β-amyloid(1–42) identify most plaque and vascular deposits in Alzheimer cortex, but not striatum

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Abstract

Alzheimer's β-peptides (Aβ) aggregation rates depend on Aβ length. We made synthetic peptide antisera to Aβ 34–40 and 37–42. Purified anti-34–40 preferentially recognizes β1–40, vascular amyloid and a subset of plaques while purified anti-37–42 recognizes Aβ1–42 and not Aβ1–40 in dot and Western blots and immunoprecipitates; 37–42 precipitates a small percentage of fibroblast secreted Aβ and strongly stains all deposits identified by monoclonals to Aβ on adjacent sections from frontal cortex, but not in dorsal striatum.

Keywords: Alzheimer's disease; β-Amyloid; Antibody; Cerebral cortex; Striatum

Deposition of a 28–43 amino acid peptide, β-protein (Aβ) accompanies Alzheimer's disease (AD) [11,12]. Aβ peptides are normally secreted by cultured cells [7,14] and found in CSF [13,14]. Sequencing from cultured cell conditioned media (CM) [5,13,16] and mass spectrographic data from CSF [13] revealed C-terminal heterogeneity with β1–40 (β40) as the major species. In contrast, in isolated amyloid deposits, β1–42 (β42) appears the major species (85–95% of plaque core Aβ) [12]. Synthetic peptide experiments show that β42 nucleates aggregation of Aβ far better than β39 or β40 [2,9]. In contrast to shorter forms, β42 tends to aggregate, is not readily cleared and accumulates in cells [10]. Collectively, these data argue that the C-terminus of Aβ may be an important variable in AD pathogenesis. To explore this hypothesis, we produced affinity purified polyclonal antibodies to Aβ37–42 which, after β40 column absorption, distinguish β42 from β40.

Peptides representing Aβ 34–40 and 37–42 with an added N-terminal lysine were synthesized on an Applied Biosystems synthesizer using Fmoc chemistry, linked to carrier with glutaraldehyde and dialyzed against 0.1 M phosphate buffer, pH 7.0. Rabbit antisera were raised as previously described [3], affinity purified on peptide agarose bead columns, and tested against synthetic β40 and β42 (Dr. Nick Ling, Whittier Institute). Affinity purified antibodies to β37–42 were absorbed on a β40 column and 20 μg/ml of aggregated β40 to remove β-conformation dependent antibodies. The β40 absorbed 37–42 antibody was used in subsequent studies ('anti-42'). The β34–40 antisera from one rabbit ('anti-40') was predominantly reactive against β40 and was specific for β40 after absorption on β42. Immunocytochemistry was performed on deparaffinized, formic acid treated 8 μM sections from 5 AD cases ranging from 71 to 92 years and 3 age-matched controls using a Vectastain kit and diaminobenzidine as described elsewhere [3]. Dr. Z. Alton of BABCO (Richmond, CA) produced monoclonal antibody 10G4 to native β40 which we have shown labels amyloid in AD and both β40 and β42 [17].

Immunoprecipitations from 10^7 cpm of 16 h conditioned media (CM) from 150 μCi TransS^{35} labeled normal human fibroblasts or Chinese hamster ovary

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Exhibit 5

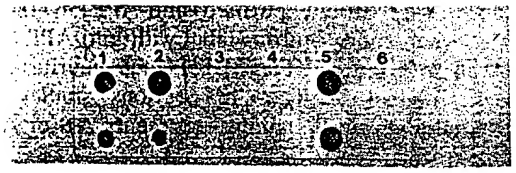

Fig. 1. Dot blot of 10 ng samples of peptides showing 1:500 and 1:1,000 dilutions of anti-42 against β42 (columns 1 and 2), or β40 (columns 3, 4), and anti-34–40 against β40 (column 5), and β42 (column 6).

cells overexpressing APP770 (CHO770, from Dr. S. Sisodia) were carried out as previously described [4].

On dot blots (Fig. 1), anti-42 recognized β42 but not β40 while the reverse was true for anti-40. On Western blots (Fig. 2A), in contrast to 10G4 anti-42 recognized β42 but not β40. Anti-40 failed to detect Aβ on Westerns. Immunoprecipitation of synthetic peptides followed by Western analysis for Aβ proved the specificity of the β42 antibody for immunoprecipitation analysis. In Fig. 2B, we show anti-42 immunoprecipitates β42, but fails to immunoprecipitate β40. With difficult and expensive direct C-terminal sequencing there is a risk of inefficient recovery and an underestimation of the percentage of β42 [5]. Having shown specificity in immunoprecipitates and optimized the recovery, we sought to detect soluble β40 and β42 in CM of labeled cells using anti-42 and affinity purified anti-34–40. In CM of both types of metabolically labeled cells, anti-42 brought down a fraction of the β40 antibody's 4 kDa Aβ (Fig. 2C). As expected, APP transfected cells produced far more 3 and 4 kDa Aβ immunoreactive material. Scanning with an LKB Ultrascan densitometer indicated that anti-42 gave 16.6% of the fibroblast Aβ 40 4 kDa counts and 18.6% of the CHO770 Aβ 40 4 kDa counts, in rough agreement with a report using a sandwich ELISA for β42 [16].

In sections from frontal cortex of 5 AD patients, β42 specific antibodies stained both plaque cores and diffuse deposits (Fig. 3) and occasional neurons, chiefly large pyramidal cells. Vascular amyloid was stained by anti-42 (Fig. 3B), but more prominently by anti-40 (Fig. 3C,G) which labeled only a subset of plaques, but very intensely. Absorption with 20 μg/ml of peptide antigen completely eliminated amyloid immunostaining (Fig. 3D–F). In contrast, absorption with β42 failed to block anti-40 staining (Fig. 3G). The observation of robust anti-42 staining of diffuse plaques in cortex shows the β42 C-terminus in one of the earliest Aβ deposits. Vessels also labeled in agreement with new sequence data [12], but anti-40 was comparatively selective for vessels (Fig. 3C).

In sharp contrast to the frontal cortex, diffuse deposits in the striatum which were readily labeled by 10G4 monoclonal (Fig. 3H) stained very weakly or not at all with anti-42 (Fig. 3I) or anti-40 (Fig. 3J). Rare anti-42 positive plaques were found, generally compact in nature, where many diffuse deposits were labeled by 10G4 monoclonal. While the more severe cases tended to have more amyloid in cortex and in the striatum, the reported phenomena were observed in all AD cases examined and normals with plaques. In agreement with other authors, despite the presence of many plaques in

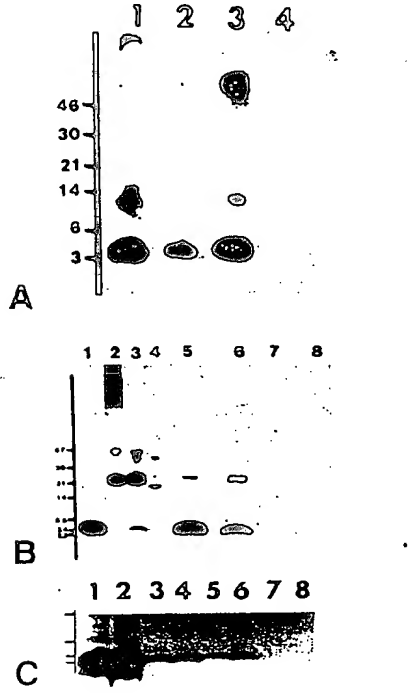

Fig. 2. A: Western analysis of 50 ng of β42 (lanes 1 and 3) and 50 ng of β40 (lanes 2 and 4) with monoclonal antibody 10G4 mapping to β5–13 (lanes 1 and 2) vs. anti-42 (lanes 3 and 4). B: immunoprecipitates of 200 ng of β40 or β42 with different antibodies detected by Western analysis with anti-Aβ monoclonal 10G4 and anti-mouse IgG peroxidase. Lane 1 = 25 ng β40 standard; lane 2 = 10G4 alone; lane 3 = 10G4 with β40; lane 4 = anti-42 with β40; lanes 5,6 = anti-42 with β42; lanes 7,8 = anti-42 absorbed with 10 μg 37–42 peptide. C: immunoprecipitates of CM from CHO770 cells or normal human fibroblasts labelled for 16 h in 1% dialzyed fetal calf serum and cysteine and methionine free medium with 300 μCi of TransS label ^{35}S (ICN, Costa Mesa, CA). Labelled media were cleared, incubated with 10 μl of antibody/ml media, and immunoprecipitates were brought down and run out on 10–20% Tris Tricine gels and autoradiographed. Mol.wts. as in B. Lanes 1, 2, 5 and 6 = CHO770. Lanes 3, 4, 7 and 8 = fibroblasts. Lanes 1–4 = affinity purified 34–40; lanes 5–8 = anti-42.

the AD dorsal striatum, only these rare compact deposits were positive for traditional amyloid stains like thioflavin S [1] and associated with fibrillar amyloid and abnormal neurites [6]. These observations are intriguing because the striatum is not associated with many neuritic plaques or significant clinical or pathological evidence of synaptic or neuronal damage [15]. We are currently investigating the possiblity that region specific Aβ metabolism results in C-terminal truncated forms of Aβ which don't readily form amyloid fibrils and are not associated with neurodegeneration.

Our anti-42 antibodies also detected selected neurons, most commonly large pyramidal cells, in AD but also normal frontal cortex (Fig. 4A). This neuronal staining contrasted sharply with the more prevalent labeling of both diffuse and mature plaques (Fig. 4B) and more closely resembled labeling by APP antibodies as shown with affinity purified anti-APP176–186 in Fig. 4C. Many more neurons label with anti-APP (Fig. 4D) than with anti-42 (Fig. 3B). Plaque neurites also labeled with anti-APP as in previous studies [3]. Whether the neuronal staining represents Aβ or APP remains unclear

APP717 mutations migrate with early onset AD and cells expressing them produce normal amounts of total Aβ, but substantially more β42 [12]. Our data show that in the cortex, the majority of diffuse and other deposits are strongly positive for Aβ42 while a subset of deposits are positive for β40 in agreement with a recent report [8]. In contrast to the cortex, in the striatum, neither the anti-42 or anti-40 show strong staining relative to more N-terminal Aβ antibodies or to staining in cortex. Collectively, these data imply that the length of Aβ is an important factor regulating

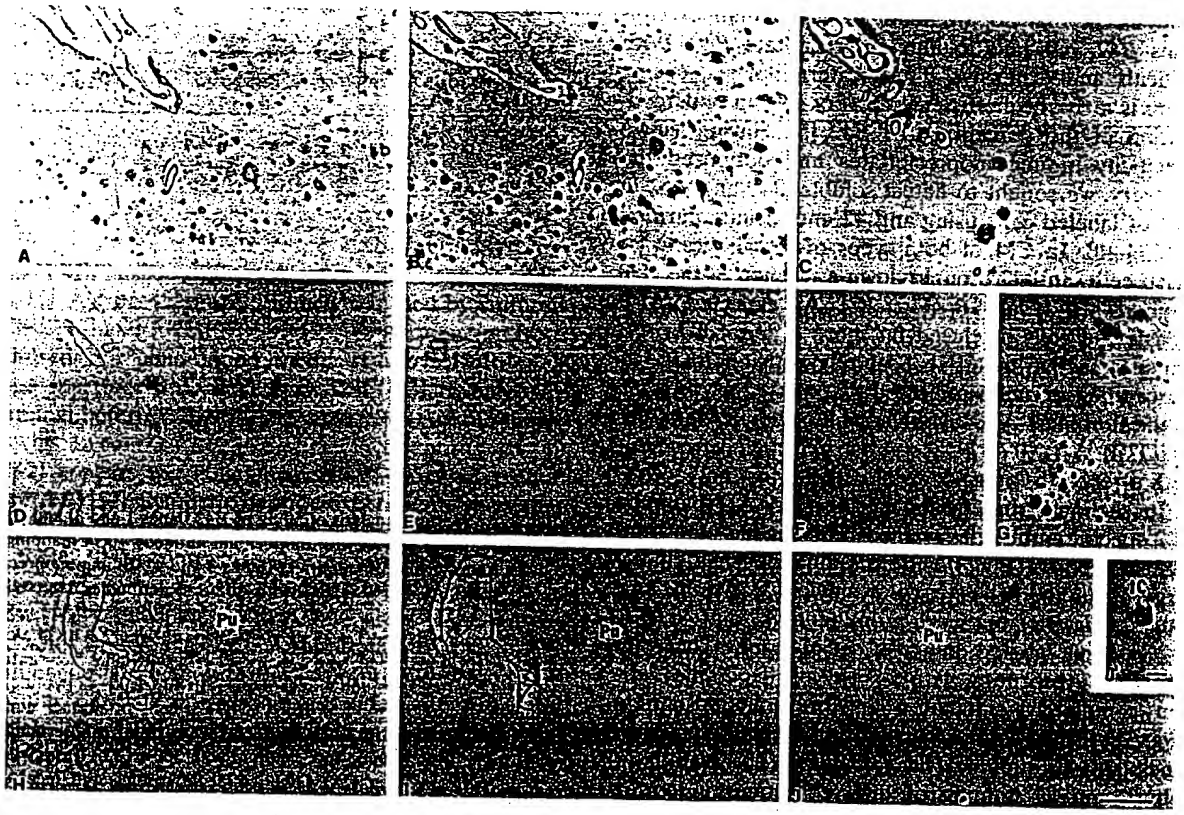

Fig. 3. A–G: AD frontal cortex immunostained with: (A) 10G; (B) 10G4 anti-42 monoclonal; (C) anti-40; (D) 10G4 absorbed β1–40; (E) anti-42 absorbed β37–42; (F) anti-40 absorbed 34–40; (G) anti-40 absorbed β1–42; (H–J) adjacent sections of putamen (Pu) with (H) 10G4, (I) anti-42, (J) anti-40. A–J: original magn. 40 ×. Bar = 250 μm. Inset J': anti-40 on insular cortex (IC) in same section as (J). Orig. magn. 100 ×. Bar = 5 μm.

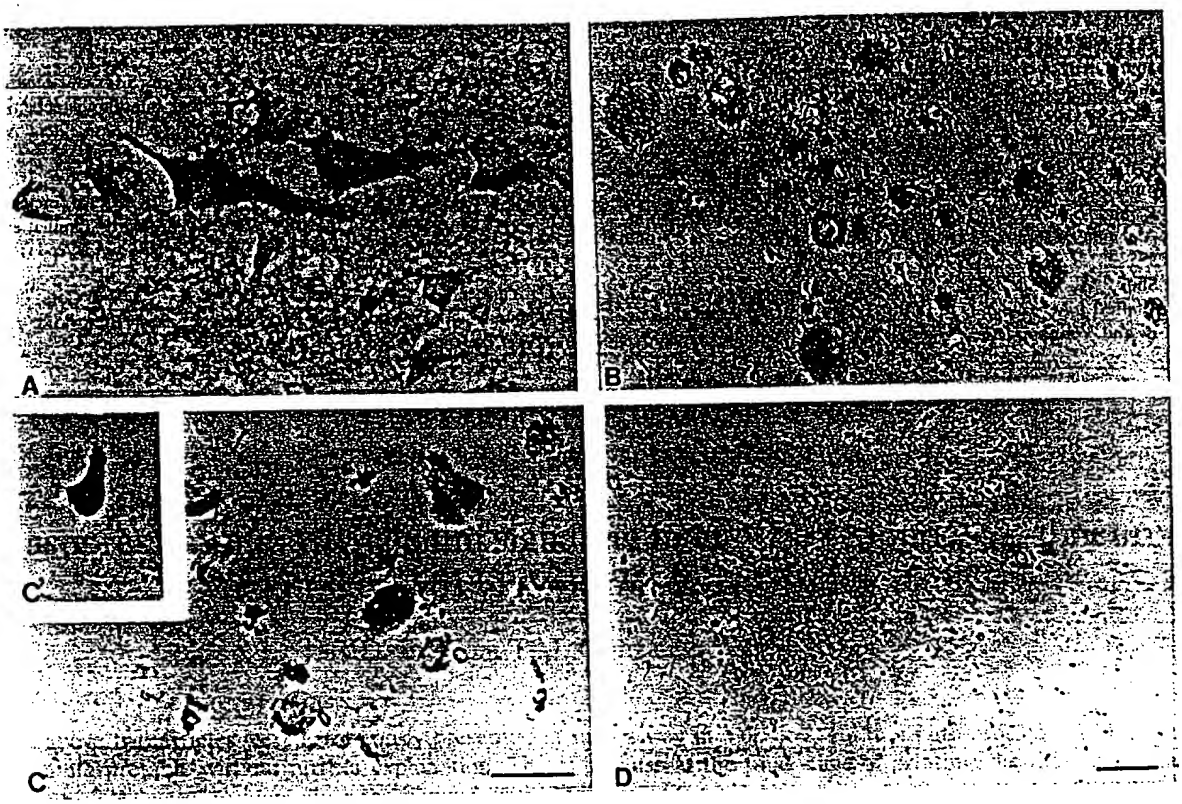

Fig. 4. A: anti-42 labeling some large pyramidal neurons in normal cortex. B: anti-42 labeling diffuse and cored plaques in AD brain. Similarly, anti-APP176–186 labeled neurons in normal (C; bar = 25 μm) and in AD (D; bar = 100 μM) cortex. B,D: orig. magn. 100 ×; A,C: orig. magn. 400 ×.

β-amyloidosis. Our data also suggest region dependent variation in Aβ deposits which may be related to plaque progression.

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